

60/087,561, filed June 1, 1998, and is a continuing application of U.S.S.N. 09/127,926, filed July 31, 1998, and 09/058,459, filed April 10, 1998.--

Please replace the paragraph beginning at page 87, line 9, with the following rewritten paragraph:

--**Table 11.** *DEE determined optimal sequences for the core positions of $G\beta 1$ as a function of $\Delta h_{0,9}^a$ --*

Please replace the paragraph beginning at page 88, line 1, with the following rewritten paragraph:

--**Table 12.** *DEE determined optimal sequences for the core positions of $G\beta 1$ as a function of $\Delta h_{1,0}^a$ --*

Please replace the paragraph beginning at page 89, line 1, with the following rewritten paragraph:

--**Table 13.** *DEE determined optimal sequences for the core positions of $G\beta 1$ as a function of $\Delta Q_{0,9}^a$ --*

Please replace the paragraph beginning at page 90, line 1, with the following rewritten paragraph:

--**Table 14.** *DEE determined optimal sequences for the core positions of $G\beta 1$ as a function of $\Delta \theta_{0,9}^a$ --*

Please replace the paragraph beginning at page 91, line 1, with the following rewritten paragraph:

--**Table 15.** *DEE determined optimal sequences for the core positions of $G\beta 1$ as a function of $\Delta \sigma_{0,9}^a$ --*

Please replace the paragraph beginning at page 91, line 21, with the following rewritten paragraph:

--The optimal sequence for the ten core positions of G β 1 that is calculated using the native backbone (i.e., no perturbation) contains three conservative mutations relative to the wild-type sequence (Table 11). Y3F and V39I are likely the result of the hydrophobic surface area burial term in the scoring function. L7I reflects a bias in the rotamer library used for these calculations. The crystal structure of G β 1 has the leucine at position 7 with a nearly eclipsed χ_2 of 111°. This strained χ_2 is unlikely to be an artifact of the structure determination since it is present in two crystal forms and a solution structure (Gronenborn et al., 1991; Gallagher et al., 1994). Our rotamer library does not contain eclipsed rotamers and no staggered leucine rotamers pack well at this position. Instead, the side-chain selection algorithm chose an isoleucine rotamer that conserves the χ_1 dihedral and is able to pack well. We expect the removal of the strained leucine rotamer to stabilize the protein, a prediction that is tested in the experimental section of this work. The sequences that result from varying individual super-secondary structure parameter values show two notable trends. Small variations in the parameter values tend to have little or no effect on the calculated sequences. For example, varying $\Delta h_{0,9}$ from -0.25 to -1.00 Å (Table 11) and $\Delta h_{1,0}$ from +0.25 to +1.25 Å (Table 2) has no effect on the calculated sequences which demonstrates the side-chain selection algorithm's tolerance to small variations in the initial backbone geometry. Large variations in the parameter values tend to result in greater sequence diversity. For example, $\Delta h_{1,0}[+1.50\text{Å}]$ contains six out of ten possible mutations relative to G β 1 (Table 12). The apparently anomalous result that occurs for $\Delta h_{0,9}$ at -1.25 and -1.50 Å, an increase in core volume, is explained by the observation that translating the helix towards the sheet plane results in creating a pocket of space in the vicinity of position 20 that ultimately leads to the observed A20V mutation.--

Please replace the paragraph beginning at page 92, line 10, with the following rewritten paragraph:

--Experimental validation of the designed cores focused on seven of the Δh -series mutants which contain between three and six sequence changes relative to G β 1. The designed sequences resulting from $\Delta\Omega$, $\Delta\theta$ and $\Delta\sigma$ perturbations are, however, in many cases identical to various Δh -series sequences. Typical far UV circular dichroism (CD) spectra are shown in Figure 15. $\Delta h_{0.9}[-1.00\text{\AA}]$, $\Delta h_{0.9}[0.00\text{\AA}]$, $\Delta h_{0.9}[+0.75\text{\AA}]$ and $\Delta h_{0.9}[+1.00\text{\AA}]$ have CD spectra that are indistinguishable from that of G β 1 while $\Delta h_{0.9}[+1.50\text{\AA}]$, $\Delta h_{1.0}[+1.50\text{\AA}]$ and $\Delta h_{0.9}[-1.50\text{\AA}]$ have CD spectra similar to that of G β 1 suggesting that all of the mutants have a secondary structure content similar to the wild-type protein. Thermal melts monitored by CD are shown in Figure 16. All of the mutants have cooperative transitions with melting temperatures (T_m 's) ranging from 53 °C for $\Delta h_{0.9}[+1.50\text{\AA}]$ to 91 °C for $\Delta h_{0.9}[0.00\text{\AA}]$ (Table 11). The T_m for G β 1 is 85°C. The measured T_m 's for $\Delta h_{0.9}[-1.50\text{\AA}]$ and $\Delta h_{0.9}[+1.50\text{\AA}]$ are for 56 residue proteins compared to 57 residue proteins in all other cases (see Methods and materials) which results in T_m 's that are estimated to be about 2 °C higher than what would be expected for the corresponding 57 residue proteins based on the T_m difference between the 56 and 57 residue versions of G β 1. The removal of the strained leucine at position seven (L7I) along with the increased hydrophobic burial generated by the Y3F and V39I mutations in $\Delta h_{0.9}[0.00\text{\AA}]$ result in a protein that is measurable more stable than wild-type G β 1. The extent of chemical shift dispersion in the 1D ^1H NMR spectrum of each mutant was assessed to gauge each protein's degree of native-like character (Fig. 5). All of the mutants, except $\Delta h_{0.9}[+1.50\text{\AA}]$, have NMR spectra with chemical shift dispersion similar to that of G β 1 suggesting that the proteins form well-ordered structures. $\Delta h_{0.9}[+1.50\text{\AA}]$